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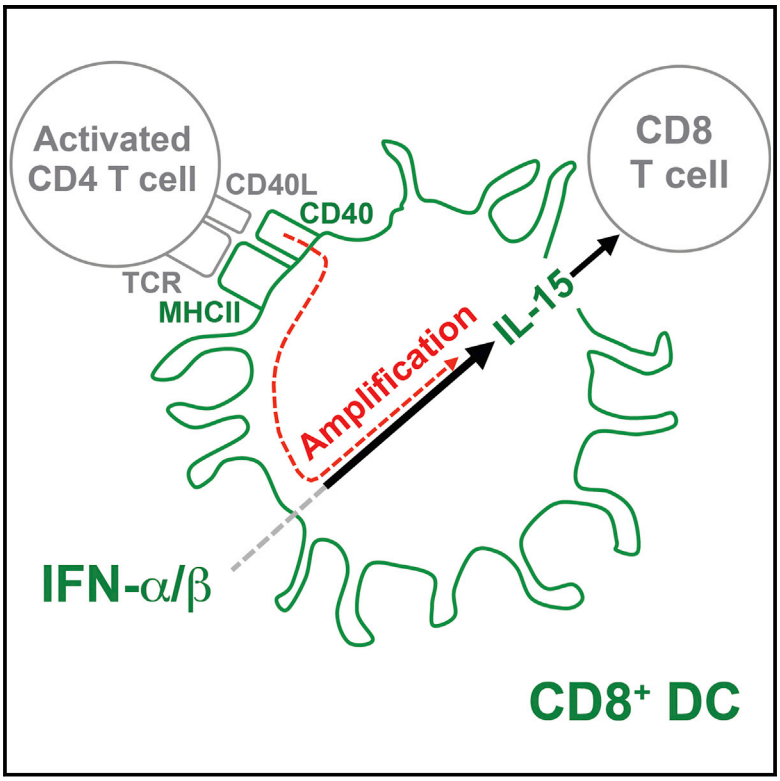
T Cell Help Amplifies Innate Signals in CD8$^+$ DCs for Optimal CD8$^+$ T Cell Priming

Graphical Abstract



Highlights

- IFN-α/β and CD4$^+$ T cells cooperate in enabling CD8$^+$ DCs to provide IL-15
- Innate circuit stimulation determines cytokine requirements of CTL priming
- T cell help for CD8$^+$ T cell priming is a means to amplify innate circuits in DCs

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In Brief

Greyer et al. show that "T cell help" optimizes CD8$^+$ T cell priming by amplifying innate signals in DCs, illustrating that the innate circuits stimulated during priming and not the CD4$^+$ T cells, dictate how T cell help is integrated into CD8$^+$ T cell priming.

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T Cell Help Amplifies Innate Signals in CD8$^+$ DCs for Optimal CD8$^+$ T Cell Priming

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SUMMARY

DCs often require stimulation from CD4$^+$ T cells to propagate CD8$^+$ T cell responses, but precisely how T cell help optimizes the priming capacity of DCs and why this appears to differ between varying types of CD8$^+$ T cell immunity remains unclear. We show that CD8$^+$ T cell priming upon HSV-1 skin infection depended on DCs receiving stimulation from both IFN-α/β and CD4$^+$ T cells to provide IL-15. This was not an additive effect but resulted from CD4$^+$ T cells amplifying DC production of IL-15 in response to IFN-α/β. We also observed that increased innate stimulation reversed the helper dependence of CD8$^+$ T cell priming and that the innate stimulus, rather than the CD4$^+$ T cells themselves, determined how "help'" was integrated into the priming response by DCs. These findings identify T cell help as a flexible means to amplify varying suboptimal innate signals in DCs.

INTRODUCTION

CD8$^+$ T cells need to clonally expand and acquire effector function to eliminate virus-infected or tumor-transformed cells. This differentiation of naive CD8$^+$ T cells into effectors is referred to as "priming" and the regulation of this process is a key function of dendritic cells (DCs). Before DCs can propagate T cell priming, they must themselves transition to a mature state that is characterized by an increased capacity to present antigen and enhanced expression of costimulatory molecules, such as CD40. Signals inducing DC maturation are typically derived from the stimulation of pattern recognition receptors and/or exposure of DCs to inflammatory mediators (Vega-Ramos and Villadangos, 2013). Interestingly, DCs often also need to integrate a signal delivered from CD4$^+$ T cells to gain full capacity to prime naive CD8$^+$ T cells (Bennett et al., 1998; Ridge et al., 1998; Schoenberger et al., 1998). This process, which is also known as "T cell help," requires that antigen-specific CD4$^+$ T cells stimulate CD40 molecules on their cognate antigen-presenting DCs (Bevan, 2004; Wiesel and Oxenius, 2012).

In considering how T cell help improves DC-induced CD8$^+$ T cell activation, it is important to appreciate that CD4$^+$ T cells are required for many, but not all, primary CD8$^+$ T cell responses. For example, CD8$^+$ T cell responses to infections with lymphocytic choriomeningitis virus (LCMV) or influenza virus can be primed in the absence of CD4$^+$ T cells (Belz et al., 2002; Wang et al., 2012). In contrast, CD8$^+$ T cell responses to other stimuli, such as infections with herpes simplex virus (HSV) (Rajasagi et al., 2009; Smith et al., 2004) or vaccinia virus (Wiesel et al., 2011) and cell-associated antigen (Behrens et al., 2004; Bennett et al., 1998) require the presence of CD4$^+$ T cells. These disparate helper requirements of CD8$^+$ T cell priming are related to the strength of the inflammatory response accompanying the acquisition of antigen (Bevan, 2004). In particular, the magnitude of the interferon-α/β (IFN-α/β) response associated with the priming process has been reported to be a determining factor, as helper-dependent CD8$^+$ T cell responses can be rendered helper independent by increasing IFN-α/β (Le Bon et al., 2003; Wiesel et al., 2011). Conversely, helper-independent LCMV-specific CD8$^+$ T cell responses become helper dependent upon abrogation of MDA5-induced IFN-α/β secretion (Wang et al., 2012). This inverse correlation between the strength of the inflammatory response and the helper dependence of the ensuing CD8$^+$ T cell response has led to the view that T cell help substitutes (Bevan, 2004; Wiesel and Oxenius, 2012) for the cues that DCs receive from innate stimuli in helper-independent situations. However, experimental validations of such a substitute role are missing and there is no coherent model that can explain why some cytotoxic T lymphocyte (CTL) responses are helper dependent while others are less so.

One idea of how T cell help could substitute for innate signals suggests that CD4$^+$ T cells regulate DC maturation when inflammatory mediators are insufficient to achieve this, proposing that CD4$^+$ T cells directly induce DC maturation via CD40 stimulation (Bevan, 2004; Cella et al., 1996). Another model envisages that

T cell help regulates the provision of cytokines that DCs need to deliver to $CD8^+$ T cells for their full effector differentiation (Wiesel et al., 2011). Notably, at least three different cytokines have been implicated here. Using a model of helper-dependent $CD8^+$ T cell-mediated allograft rejection, it was reported that $CD4^+$ T cells were required to induce interleukin-12 (IL-12) secretion by DCs (Filatenkov et al., 2005). In the context of vaccinia virus infections, T cell help was suggested to regulate the provision of IFN-α/β from DCs to vaccinia virus-specific $CD8^+$ T cells (Wiesel et al., 2011), whereas $CD4^+$ T cells are believed to be required for IL-15 provision by DCs during helper-dependent adenoviral vaccine-specific $CD8^+$ T cell responses (Oh et al., 2008). While these studies generally support the idea that T cell help operates at the level of cytokine provision by DCs, they raise the intriguing question of how the one signal (i.e., T cell help) can lead to such differing cytokine requirements of the resulting $CD8^+$ T cell response.

Given the above, we set out to systematically investigate how T cell help regulates $CD8^+$ T cell priming. Contrasting with the current idea that T cell help substitutes for innate signals (Bevan, 2004; Wiesel and Oxenius, 2012), our findings are consistent with a view of T cell help in which $CD4^+$ T cells amplify innate signals that alone are insufficient to convert poorly immunogenic DCs into those capable of effective priming. This is an important distinction to the current model (Bevan, 2004; Wiesel and Oxenius, 2012), as it entails that the innate signals associated with a particular antigen determine the cytokine(s) that ultimately mediate $CD8^+$ T cell immunity and that T cell help simply serves to amplify this response. Importantly, viewing T cell help as a means to amplify innate signals explains why the "help" provided by $CD4^+$ T cells can appear to be mediated via differing cytokine responses, yet be delivered by the same mechanism, i.e., CD40 stimulation.

RESULTS

$CD8^+$ T Cell Response to HSV-1 Skin Infection Requires T Cell Help

To dissect how T cell help improves the capacity of DCs to prime antigen-specific $CD8^+$ T cell responses in vivo, we employed a model of peripheral HSV-1 infection (Bedoui et al., 2009; Davey et al., 2010; van Lint et al., 2004). We first validated that the $CD8^+$ T cell response to epicutaneous HSV-1 infection was helper dependent as implied by our earlier work using different routes of virus inoculation (Smith et al., 2004). For this, we infected two different cohorts of $CD4^+$ T cell-deficient mice with HSV-1 on the skin and assessed $CD8^+$ T cell priming 7 days later in the spleen, a time point that represents the peak of the priming response (Coles et al., 2002; Davey et al., 2010). Both anti-CD4 antibody-treated and MHC-II-deficient mice (*H2-ab1*$^{-/-}$) mounted impaired $CD8^+$ T cell responses following HSV-1 skin infection as assessed by expansion of $CD8^+$ T cells specific for the immunodominant epitope $gB_{498–505}$ derived from HSV-1 glycoprotein B (Figure 1A). Together with the demonstration that mice deficient in CD40L (*CD40lg*$^{-/-}$) or CD40 (*CD40*$^{-/-}$) had similarly impaired primary HSV-specific $CD8^+$ T cell responses (Figure 1B), these findings demonstrated that the $CD8^+$ T cell response to skin HSV-1 infection required $CD4^+$ T cells and CD40L-CD40 interactions.

T Cell Help Is Not Required for DC Maturation following HSV-1 Skin Infection

We considered the proposal that $CD4^+$ T cells induce DC maturation in helper-dependent scenarios (Bevan, 2004). To first determine how accurately in vivo DC maturation could be measured in our model, we isolated DCs from the brachial lymph node that drains the site of HSV-1 skin infection 2 days later and analyzed the DC compartment for surface markers classically associated with DC maturation, including MHC-II and CD40 (Reis e Sousa, 2006). We focused on lymph node resident $CD8^+$ DCs (Figure 1C), as our previous studies have revealed this subset of DCs to initiate $CD8^+$ T cell priming in response to HSV-1 skin infection (Allan et al., 2003; Bedoui et al., 2009). $CD8^+$ DCs from HSV-1 infected mice had increased surface expression of MHC-II and CD40 relative to $CD8^+$ DCs from uninfected mice (Figures 1D and 1E). This increase in MHC-II and CD40 expression was not due to contaminating migratory DCs in the $CD8^+$ DC gate, as migratory DCs do not express CD8 (Bedoui et al., 2009). To validate that these phenotypic changes represented biologically relevant DC maturation that enabled the priming of $CD8^+$ T cells, we isolated MHC-II^{high} and MHC-II^{low} $CD8^+$ DCs (Figure 1F) from the brachial lymph node of mice infected 2 days earlier on the skin with HSV-1. These DCs were co-incubated with 50,000 naive, Carboxyfluorescein succinimidyl ester (CFSE)-labeled transgenic $CD8^+$ gBT-I cells specific for a H-$2K^b$-restricted epitope from glycoprotein B. Strikingly, only MHC-II^{high} $CD8^+$ DCs induced gBT-I proliferation (Figure 1F), confirming that the activated in vivo MHC-II^{high} phenotype correlated with the capacity of $CD8^+$ DCs to effectively stimulate naive HSV-specific $CD8^+$ T cells. Having confirmed MHC-II upregulation as a surrogate marker for in vivo DC activation, we tested the suggestion that $CD4^+$ T cells contributed to DC activation (Bevan, 2004). However, when comparing the ability of $CD8^+$ DCs to increase MHC-II and CD40 expression in response to HSV-1 skin infection, it was evident that DC activation occurred regardless of the presence of $CD4^+$ T cells (Figures 1G and 1H). These findings indicated that the contribution of $CD4^+$ T cells to HSV-specific $CD8^+$ T cell priming was not linked to the upregulation of MHC-II and/or CD40 expression by $CD8^+$ DCs.

IFN-α/β and IL-15 Are Crucial for Effective HSV-Specific $CD8^+$ T Cell Priming

Given that T cell help was not required for the increased expression of MHC-II and CD40 by $CD8^+$ DCs associated with HSV-1 skin infection, we next considered the possibility that T cell help could enhance the capacity of DCs to deliver cytokines to the $CD8^+$ T cells. We focused on IL-12, IFN-α/β, and IL-15, as these have been suggested to play a role in other helper-dependent types of $CD8^+$ T cell responses (Filatenkov et al., 2005; Oh et al., 2008; Wang et al., 2012; Wiesel et al., 2011). We infected the relevant cytokine/cytokine receptor-deficient mice with HSV-1 on the skin and determined the magnitude of the HSV-specific $CD8^+$ T cell response 7 days later. While we observed no difference in HSV-specific $CD8^+$ T cell priming in *Il12b*$^{-/-}$ mice (Figure S1), we noted that the priming of endogenous HSV-specific $CD8^+$ T cells, as measured by H2-K^b-restricted $gB_{498–505}$ tetramers and intracellular cytokine staining for IFN-γ was impaired in mice lacking the IFN-α/β receptor (IFNαR2)

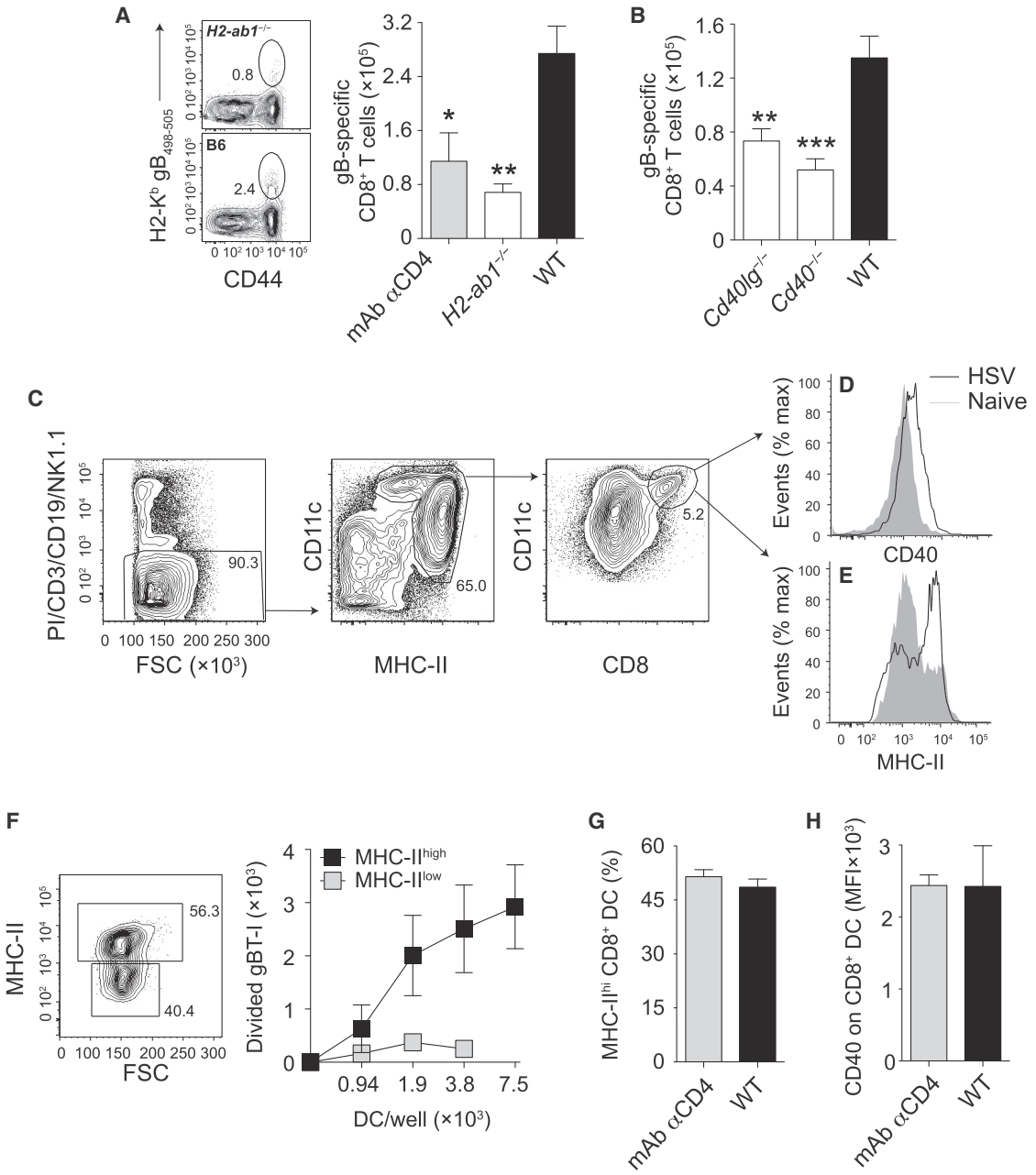


Figure 1. CD4$^+$ T Cells Are Required for HSV-Specific CD8$^+$ T Cell Priming, but Not for CD8$^+$ DC Activation

(A and B) Representative plots and absolute number of $gB_{498–505}$-specifc CD8$^+$ T cells in the spleen of CD4$^+$ T cell-deficient mice and wild-type B6 mice (WT) (A) and *Cd40lg*$^{-/-}$, *Cd40*$^{-/-}$, and WT mice (B) infected with HSV-1 on flank skin 7 days earlier.

(C–E) Gating strategy (C) and CD40 (D) and MHC-II (E) surface expression on CD8$^+$ DCs enriched from the brachial lymph node of mice infected on flank skin 2 days earlier with HSV-1 compared to CD8$^+$ DCs from skin-draining lymph nodes of uninfected naive controls.

(F) Sort profiles of MHC-II^{high} CD8$^+$ DCs (black squares) and MHC-II^{low} CD8$^+$ DCs (gray squares) from the brachial lymph nodes of mice infected on the skin with HSV-1 2 days earlier. Absolute numbers of proliferated, CFSE-labeled gBT-I upon co-incubation with serial dilutions of CD8$^+$ DCs for 60 hr are shown.

(G and H) Percent of MHC-II^{high} CD8$^+$ DCs (G) and mean fluorescence intensity of CD40 expression on CD8$^+$ DCs (H) from CD4-depleted and WT mice infected 2 days earlier with HSV-1 on flank skin.

All data are pooled results from at least two independent experiments (n = 5 per experiment) and are expressed as mean + SEM. Asterisks indicate statistically significant differences versus controls as assessed by one-way ANOVA (A and B); *p < 0.05; **p < 0.001; ***p < 0.0001.

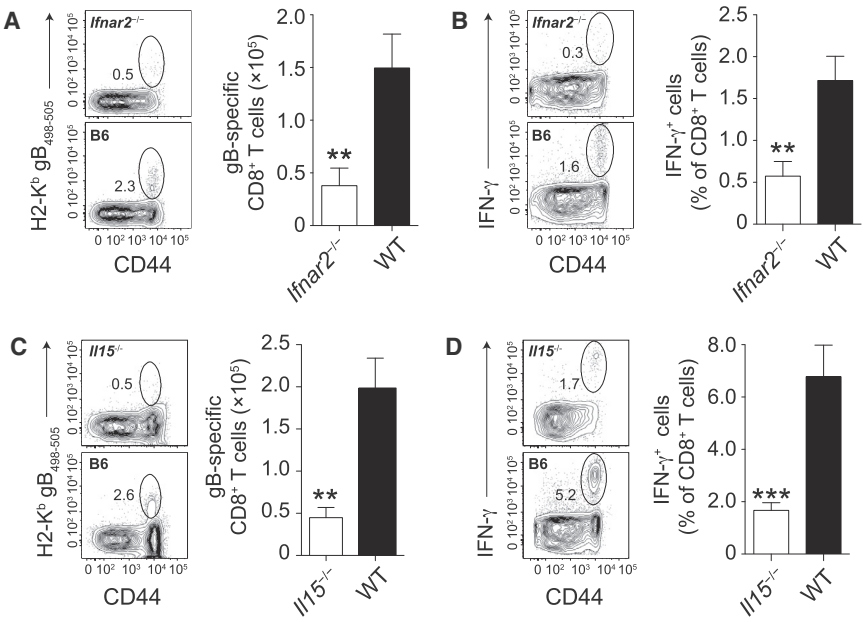


Figure 2. IFN-α/β and IL-15 Are Required for HSV-Specific CD8⁺ T Cell Priming

(A–D) Representative plots and absolute number of $gB_{498–505}$-specifc CD8⁺ T cells (A and C) and frequency of IFN-γ producing CD8⁺ T cells in the spleen of *Ifnar2*$^{-/-}$, *Il15*$^{-/-}$, and WT mice infected with HSV-1 on flank skin 6 or 7 days earlier (B and D). Data are pooled from at least three independent experiments (n = 5 per experiment) and expressed as mean + SEM. Asterisks indicate statistically significant differences versus controls as assessed by Student's t test; **p < 0.001; ***p < 0.0001.

(Figures 2A and 2B) or IL-15 (Figures 2C and 2D). Figures S2A and S2B show background staining with H2-K^b-restricted $gB_{498–505}$ tetramers and intracellular staining for IFN-γ in mock-infected mice. These findings demonstrated that HSV-specific CD8⁺ T cell priming depended on both IFN-α/β and IL-15, but not IL-12.

DC-Derived IL-15 Is Required for In Vivo Priming of HSV-Specific CD8⁺ T Cells

If IFN-α/β and IL-15 acted as relevant signals in the direct interaction between DCs and HSV-specific CD8⁺ T cells, then DCs should be the relevant source of these cytokines and the CD8⁺ T cells should respond directly to them. We first tested whether DCs were required to provide the IL-15. To this end, we reconstituted irradiated mice with a 1:1 mix of bone marrow from *Il15*$^{-/-}$ mice and CD11c-DTR transgenic mice. The latter mice express a highly sensitive diphtheria toxin receptor (DTR) that is primarily expressed by DCs (Jung et al., 2002). After about 8 weeks of reconstitution, these chimeras contained IL-15-competent DCs from the CD11c-DTR bone marrow and IL-15-deficient DCs derived from the *Il15*$^{-/-}$ bone marrow. Diphtheria toxin (DTx) treatment of these chimeras selectively eliminated IL-15-competent DCs, but not IL-15-deficient DCs, resulting in mice where IL-15 was selectively missing from DCs. Notably, DTx-treated chimeras infected with HSV-1 on the skin were impaired in CD8⁺ T cell expansion (Figure 3A). The data are expressed as relative change to control chimeras, because the magnitude of the T cell responses in chimeras varied between experiments. This impairment in CD8⁺ T cell priming was not due to an overall reduction in DCs, as control chimeras in which wild-type DCs remained after DTx treatment mounted CD8⁺ T cell responses that were indistinguishable from untreated controls (Figure S3). These finding indicated that DCs were the relevant source of IL-15 required for efficient in vivo HSV-specific CD8⁺ T cell priming.

DCs, but Not HSV-Specific CD8⁺ T Cells, Require IFN-α/β Stimulation

We next addressed whether IFN-α/β also acted as a crucial signal in the direct interaction between DCs and the CD8⁺ T cells. Considering the ubiquitous expression of IFNαR2, we first determined whether immune cells were generally required to express IFNαR2 for optimal HSV-specific CD8⁺ T cell priming through the use of bone marrow chimeras. We observed that virus-specific CD8⁺ T cell priming in chimeras infected with HSV-1 on the skin was only impaired if IFNαR2 was absent from the radiosensitive bone-marrow-derived compartment (Figure 3B), indicating that immune cells needed to express IFNαR2. To interrogate whether the CD8⁺ T cells required IFNαR for optimal priming, we crossed the gBT-I line onto the *Ifnar2*$^{-/-}$ background and transferred wild-type gBT-I and *Ifnar2*$^{-/-}$ gBT-I into B6 mice 1 day before infection with HSV-1 on the skin. Interestingly, gBT-I cells expanded in response to the infection regardless of whether they expressed IFNαR2 or not (Figure 3C), indicating that the CD8⁺ T cells did not require direct stimulation by IFN-α/β. We reasoned that DCs might instead be targeted by IFN-α/β and tested this in mixed bone marrow chimeras reconstituted with a 1:1 mix of bone marrow from *Ifnar2*$^{-/-}$ mice and CD11c-DTR transgenic mice. In support of this, DTx-treated chimeras infected with HSV-1 on the skin were impaired in HSV-specific CD8⁺ T cell expansion (Figure 3D), indicating that IFNαR2 expression by CD11c^{high} cells was required for efficient CD8⁺ T cell priming. A picture thus emerged in which the helper-dependent priming of HSV-specific CD8⁺ T cells required both IFN-α/β and IL-15, yet only IL-15 had to be provided from DCs to the CD8⁺ T cells, while IFN-α/β was required further upstream (Figure 3E).

Enhanced IL-15 Production by Lymph-Node-Resident CD8⁺ DCs after HSV-1 Skin Infection

To better understand this functional relationship between IL-15 and IFN-α/β, we examined IL-15 expression in CD8⁺ DCs, which as mentioned above are key to the initiation of HSV-specific CD8⁺ T cell priming (Bedoui et al., 2009). Interestingly, CD8⁺ DCs sorted from the brachial lymph node of mice infected on the skin with HSV-1 2 days earlier increased the relative expression of *Il15* when compared to those obtained from uninfected

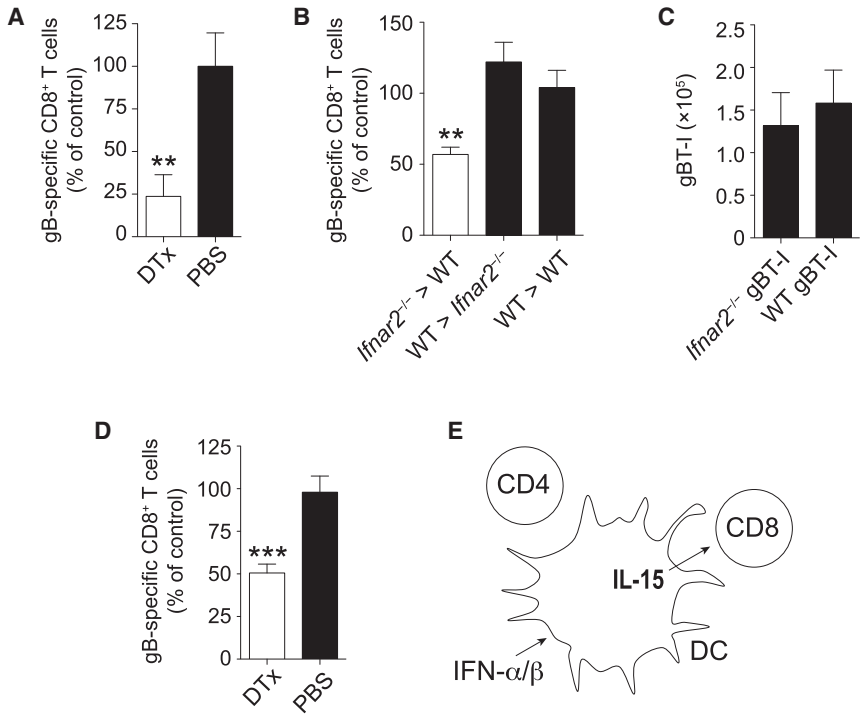


Figure 3. DCs Require Stimulation from IFN-α/β and Provide IL-15 to HSV-Specific CD8⁺ T Cells

(A) Relative proportion of $gB_{498-505}$-specifc CD8⁺ T cells in the spleen of chimeric mice containing a 1:1 mix of CD11c DTR and *Il15*$^{-/-}$ bone marrow, treated with DTx or PBS and infected with HSV-1 on flank skin 7 days earlier.

(B) Relative proportion of $gB_{498-505}$-specifc CD8⁺ T cells in the spleen of WT or *Ifnar2*$^{-/-}$ mice reconstituted with *Ifnar2*$^{-/-}$ or WT bone marrow 7 days after HSV-1 skin infection.

(C) Absolute number of WT and IFNαR2-deficient gBT-I cells in the spleen on day 7 after infection of B6 mice transferred with 2.5 × 10^4 naive WT or IFNαR2-deficient gBT-I 1 day prior to infection with HSV-1 on flank skin.

(D) Relative proportion of $gB_{498-505}$-specifc CD8⁺ T cells in the spleen of chimeric mice containing a 1:1 mix of CD11c DTR and *Ifnar2*$^{-/-}$ bone marrow, treated with DTx or PBS, and infected with HSV-1 on flank skin 7 days earlier.

(E) Helper-dependent priming of HSV-specific CD8⁺ T cells required both IFN-α/β and IL-15, yet only IL-15 had to be provided from DCs to the CD8⁺ T cells, while IFN-α/β was required upstream of this.

Data are pooled from at least two independent experiments (n ≥ 5 per experiment) and are expressed as mean + SEM. In the case of chimeras, data are expressed as percent change of control chimeras (CD45.1 > WT). Asterisks indicate statistically significant differences versus controls as assessed by Student's t test (A and D) and one-way ANOVA (B); **p < 0.001; ***p < 0.0001.

mice (Figure 4A). To dissect whether this increase in IL-15 transcription was uniform among the CD8⁺ DCs or specific to only some, we examined surface expression of the α chain of the IL-15 receptor, which is required for trans-presentation of IL-15 (Stonier and Schluns, 2010). Consistent with the transcription data, we detected an increase in IL-15Rα surface protein expression on MHC-II^{hi} CD8⁺ DCs isolated from mice infected 2 days earlier on the skin with HSV-1 (Figure 4B). Intriguingly, this increase was restricted to CD8⁺ DCs that had upregulated MHC-II expression (Figure 4C), highlighting that the induction of IL-15 was specific to only some of the CD8⁺ DCs in that lymph node. These findings indicated that lymph-node-resident CD8⁺ DCs increased IL-15/IL-15Rα expression in response to HSV-1 skin infection and that this increase was a distinct function of CD8⁺ DCs that have acquired an activated phenotype. That these helper-dependent changes in IL-15 production by CD8⁺ DCs occurred within 48 hr after HSV-1 skin infection also aligns with the expected kinetics of T cell help, which is delivered within the first 24–72 hr after antigen encounter (Mempel et al., 2004; Shedlock and Shen, 2003; Smith et al., 2004).

T Cell Help and IFN-α/β Cooperate to Allow CD8⁺ DCs to Provide IL-15

Equipped with a better understanding of IL-15 regulation in CD8⁺ DCs following HSV-1 skin infection, we sought to resolve how these processes were linked to the IFN-α/β-dependent nature of the HSV-specific CD8⁺ T cell response and its requirement for T cell help. With DCs requiring stimulation through IFN-α/β (Figure 3D) and previous demonstrations showing that IFN-α/β can induce IL-15 (Mattei et al., 2001), we questioned whether IFNαR stimulation was required for the provision of IL-15 by CD8⁺ DCs. Indeed, when we sorted CD8⁺ DCs from HSV-1-infected *Ifnar2*$^{-/-}$ mice, we noted that the increase in IL-15/IL-15Rα expression was no longer evident (Figures 4A–4C). To also resolve how these processes were linked to the helper-dependent nature of the HSV-specific CD8⁺ T cell response, we assessed whether the increase in IL-15/IL-15Rα expression required CD4⁺ T cells. Interestingly, the increase in IL-15/IL-15Rα expression by CD8⁺ DCs from HSV-1 infected mice was also abrogated when infected mice were depleted of CD4⁺ T cells (Figures 4A–4C). These results indicated that the in vivo provision of IL-15 by CD8⁺ DCs that is required for effective CD8⁺ T cell priming depended on the DCs receiving both T cell help and stimulation by IFN-α/β (Figure 4D). These findings also established that neither T cell help nor IFN-α/β stimulation alone were sufficient to increase IL-15 expression by CD8⁺ DCs in the context of HSV-1 skin infection.

CD40 Stimulation Amplifies IFN-α-Induced IL-15 Production

The above findings indicate an intriguing synergy in the regulation of IL-15 between T cell help and IFN-α/β signals. To better understand how these signals cooperated in allowing DCs to provide IL-15, we employed an in vitro system in which equivalents of CD8⁺ DCs (eCD8⁺ DCs), the DC subset responsible for in vivo priming of HSV-specific CD8⁺ T cells (Bedoui et al., 2009), can be grown from bone marrow cells by exposure to FMS-like tyrosine kinase 3 ligand (flt3L) (Naik et al., 2005). We sorted eCD8⁺ DCs on the basis of SIRPα^{low} CD24^{hi} expression on day 8 from these cultures and stimulated them in vitro with

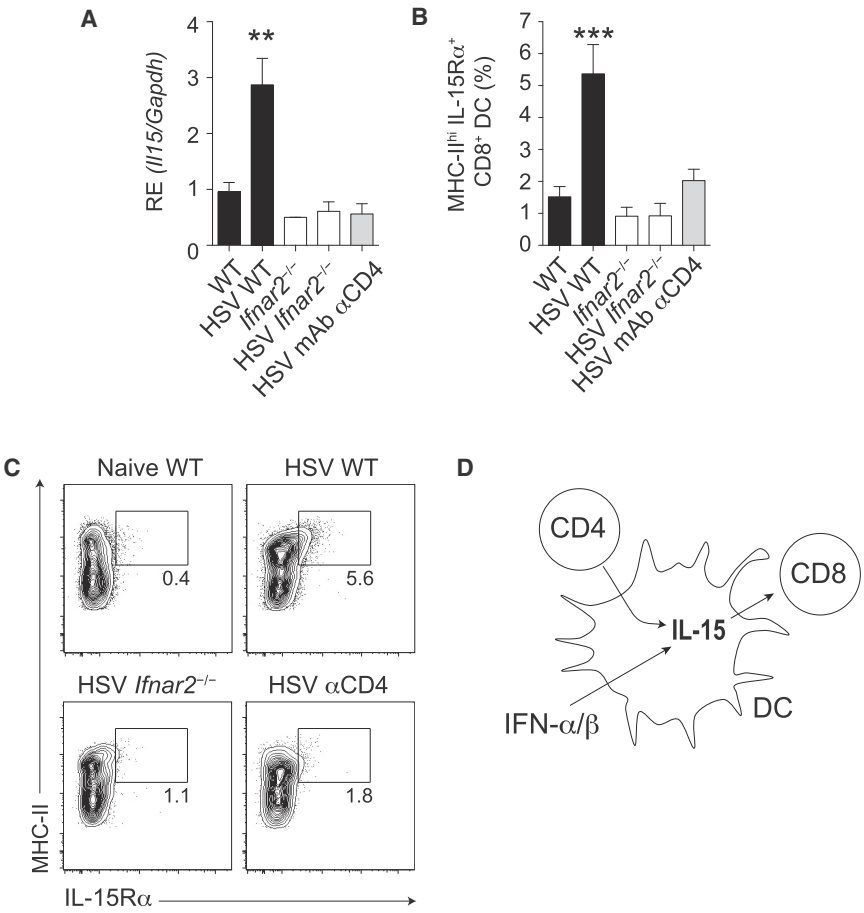


Figure 4. T Cell Help and IFN-α/β Cooperate to Allow CD8⁺ DCs to Provide IL-15

(A) Relative expression (RE) of *Il15* by CD8⁺ DCs sorted from lymph nodes of uninfected controls or *Ifnar2*$^{-/-}$, anti-CD4 mAb-treated, and WT mice infected 2 days earlier on flank skin with HSV-1.

(B and C) Frequency (B) and representative plots (C) of IL-15Rα surface protein expression by MHC-II^{hi} CD8⁺ DCs sorted from lymph nodes of uninfected controls or *Ifnar2*$^{-/-}$, anti-CD4 mAb-treated, and WT mice infected 2 days earlier on flank skin with HSV-1. Contour plots in (C) are concatenated from four individual experiments.

(D) In vivo provision of IL-15 by CD8⁺ DCs required for effective CD8⁺ T cell priming depended on the DCs receiving both T cell help and stimulation by IFN-α/β.

Data are pooled from at least three independent experiments (n = 2–5 per experiment) and expressed as mean + SEM. Real-time PCR data are expressed relative to *gapdh* expression. Asterisks indicate statistically significant differences versus controls as assessed by one-way ANOVA (A and B); ***p < 0.0001.

recombinant murine IFN-α A in the presence or absence of a stimulating anti-CD40 monoclonal antibody (FGK45) that mimics T cell help (Bennett et al., 1998; Feau et al., 2011; Schoenberger et al., 1998). When we assessed relative expression of *Il15* by eCD8⁺ DCs stimulated for 4 hr with escalating doses of IFN-α A using real-time PCR, we observed that IFN-α A in the doses tested hardly induced any IL-15 expression (Figure 5A). Intriguingly, when IFN-α A was administered together with the T cell help mimicking anti-CD40 antibody, 1,000 U/ml IFN-α A induced substantial IL-15 expression in the DC (Figure 5A). Notably, we also observed a similar pattern of CD40-induced IL-15 amplification when we stimulated freshly isolated CD8⁺ DCs with IFN-α A ex vivo (Figure 5B). Importantly, this synergy was not due to an additive effect between the two stimuli, as the anti-CD40 mAb alone did not induce IL-15 in the DCs, even when tested at higher doses than those used in combination with IFN-α A (data not shown). This synergistic activity was also not a consequence of CD40-dependent IFN-α/β secretion from DCs that could potentially act in an autocrine fashion to augment the original signal, as the anti-CD40 mAb failed to elicit any IFN-α/β when applied alone and also did not amplify CpG-induced IFN-α/β secretion (data not shown). These findings demonstrated that CD40 stimulation amplified the capacity of CD8⁺ DCs to provide IL-15 in response to suboptimal IFN-α/β stimulation.

Increasing the Strength of Innate Stimulation Reverses the Helper Dependence of CD8⁺ T Cell Priming

When considered in the context of the well-established observation that T cell help for CD8⁺ T cell priming is indispensable when inflammatory signals associated with the response are weak (Bevan, 2004; Wiesel and Oxenius, 2012), the above findings imply that T cell help serves to amplify IFN-α/β signals that alone are insufficient to elicit the required IL-15 response by DCs. To further investigate this possibility and to validate findings from our HSV-1 skin infections in another model of helper-dependent CD8⁺ T cell priming, we exposed mice to cell-associated ovalbumin (OVA) and determined how the strength of an accompanying IFN-α/β signal would affect the helper-requirement of the ensuing endogenous OVA-specific CD8⁺ T cell response. We chose Poly(I:C) as the inflammatory stimulus due to its potent ability to elicit IFN-α/β secretion in vivo and its common use as an adjuvant to stimulate DCs (Soares et al., 2007). Consistent with our earlier work (Bennett et al., 1998), cell-associated OVA induced a CD8⁺ T cell response in the spleen 7 days after antigen challenge that had a distinct helper dependence (shown as relative change in Figure 5C and as absolute numbers in Figure S4). Interestingly, this helper dependence became less stringent with increasing amounts of Poly(I:C) delivered at the time of antigen provision (Figure 5C). Critically, although the helper dependence of the response could be reversed by increasing the strength of the Poly(I:C) stimulus, the OVA-specific CD8⁺ T cell response depended on IL-15 regardless of whether it required T cell help or not (Figure 5D). These observations extended the importance of T cell help amplified IL-15 to another type of helper-dependent CD8⁺ T cell priming and

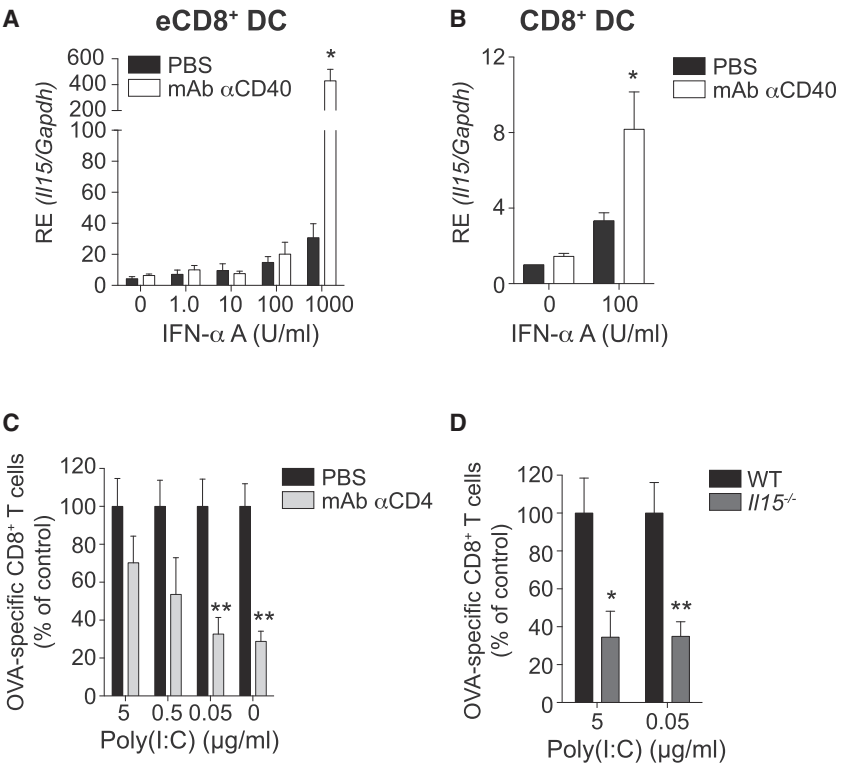


Figure 5. T Cell Help Amplifies IFN-α/β-Induced IL-15

(A) Relative expression (RE) of *Il15* by eCD8$^+$ DCs sorted from flt3L-stimulated B6 bone marrow cultures stimulated with escalating concentrations of IFN-α A in the presence (open squares) or absence (closed squares) of anti-CD40 mAb.

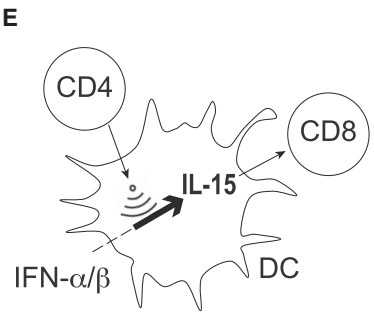
(B) RE of *Il15* by freshly isolated splenic XCR1$^+$ CD8$^+$ DCs stimulated with IFN-α A in the presence (open squares) or absence (closed squares) of anti-CD40 mAb.

(C) Relative expansion of OVA-specific CD8$^+$ T cells in the spleen 7 days after injection of cell-associated OVA in the presence of various doses of Poly(I:C) into CD4-depleted and PBS-treated WT mice.

(D) Relative expansion of OVA-specific CD8$^+$ T cells in the spleen 7 days after injection of cell-associated OVA in the presence of 5 μg and 0.05 μg Poly(I:C) into *Il15*$^{-/-}$ and WT mice.

(E) CD4$^+$ T cells amplify the IFN-α/β-induced provision of IL-15 by CD8$^+$ DCs to CD8$^+$ T cells responding to cognate antigen.

Data are pooled from two independent experiments (n = 2–5 per experiment) and are expressed as mean + SEM. Relative expansion is expressed as percent change of WT control. Real-time PCR data are expressed relative to *gapdh* expression. Asterisks indicate statistically significant differences versus controls as assessed by Student's t test (A and C) or one-way ANOVA (B); *p < 0.05; **p < 0.001.



indicated that elicitation of the IL-15 pathway only depended heavily upon T cell help when the innate signal inducing this pathway was limiting.

CD40 Stimulation Amplifies IFN-α4-Induced Chemokine and IL-6 Production

Rather than CD4$^+$ T cells delivering a distinct signal to DCs that substitutes for absent or insufficient innate stimulation (Bevan, 2004; Wiesel and Oxenius, 2012), our observations identify T cell help as a means to amplify the efficiency with which IFN-α/β elicit IL-15 in DCs (Figure 5E). These observations not only suggest that the relevance of T cell help should be seen as a function of the strength of the IFN-α/β signal but also raise the intriguing question of whether CD4$^+$ T cells can amplify other molecules induced through IFN-α/β. To address this, we analyzed the supernatants from IFN-α A-stimulated eCD8$^+$ DCs for molecules that have been associated with T cell help for CD8$^+$ T cell priming. While IL-12p40 (Filatenkov et al., 2005) could not be detected in response to IFN-α A (data not shown), this stimulus evoked a dose-dependent increase in CCL3 and CCL4, chemokines that both have been previously shown to play an important role in the spatiotemporal regulation of T cell help (Castellino et al., 2006) (Figure 6A). Stimulating the DCs with IFN-α A in the presence of anti-CD40 mAb further enhanced CCL4 secretion (Figure 6A). Interestingly, when we measured two other molecules that have been discussed in the context of T cell help, namely IL-6 (Castellino and Germain, 2007) and CCL5 (Crawford et al., 2011), we noticed a pattern, in which IFN-α A alone was barely capable of inducing IL-6 and CCL5 at the concentrations tested (Figure 6A). However, when assisted by the anti-CD40 mAb, these doses of IFN-α A now induced IL-6 and CCL5 secretion from the DCs (Figure 6A). Comparable to the effects on IL-15 (Figure 5A), the amplifying effect of CD40 was not simply an additive effect between IFN-α A and CD40 stimulation, as the anti-CD40 mAb alone did not induce any of these factors. These findings demonstrated that mimicking T cell help in vitro increased the sensitivity and capacity of eCD8$^+$ DCs to produce a wider range of IFN-stimulated downstream mediators, indicating that T-cell-help-mediated amplification of innate signals was not unique to the IFN-α/β-IL-15 axis.

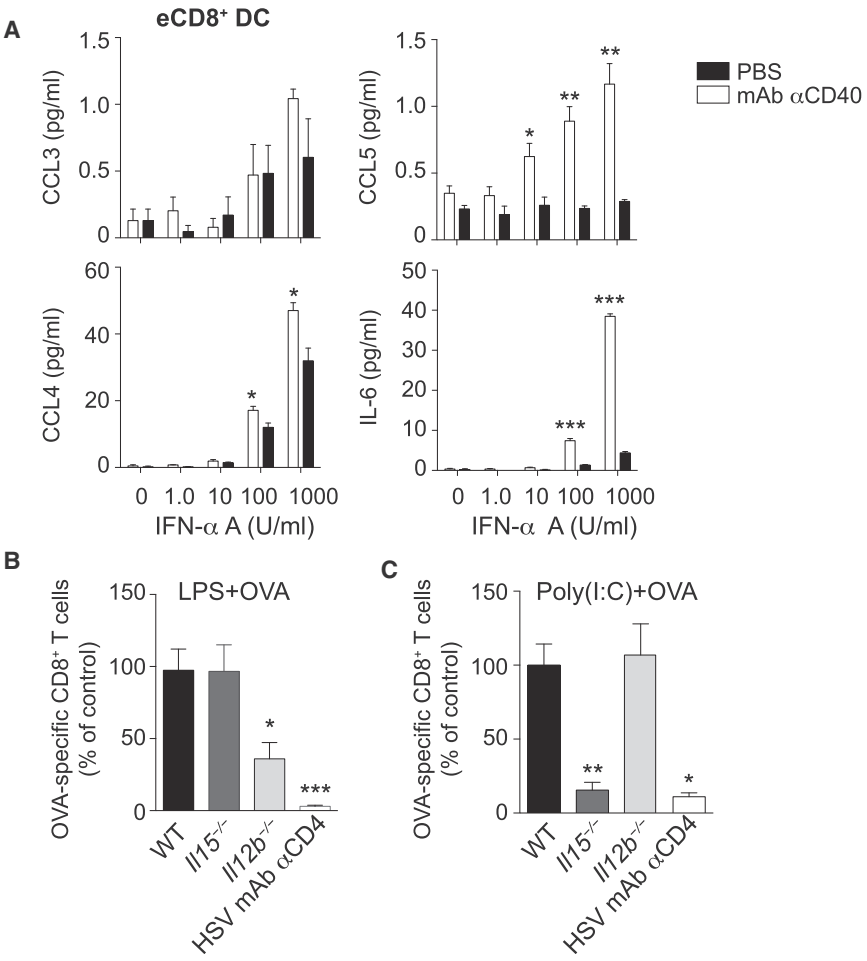


Figure 6. The Innate Stimulus Determines How T Cell Help for CD8$^+$ T Cell Priming Is Mediated

(A) CCL3, CCL4, CCL5, and IL-6 protein levels in the supernatants of eCD8$^+$ DCs sorted from flt3L-stimulated bone marrow cultures stimulated with escalating concentrations of IFN-α A in the presence (open squares) or absence (closed squares) of anti-CD40 mAb for 4 hr.

(B) Relative expansion of OVA-specific CD8$^+$ T cells in the spleen 7 days after injection of cell-associated OVA in the presence of 1 μg LPS into *Il15*$^{-/-}$, *Il12b*$^{-/-}$, CD4-depleted, and WT mice.

(C) Relative expansion of OVA-specific CD8$^+$ T cells in the spleen 7 days after injection of cell-associated OVA in the presence of 0.05 μg Poly(I:C) into *Il15*$^{-/-}$, *Il12b*$^{-/-}$, CD4-depleted, and WT mice.

Data are pooled from at least two independent experiments (n = 5 per experiment) and are expressed as mean + SEM. Relative expansion is expressed as percent change of WT control. Asterisks indicate statistically significant differences versus controls as assessed by Student's t test (A) and one-way ANOVA (B and C); *p < 0.05; **p < 0.001; ***p < 0.0001.

The Innate Stimulus Determines How T Cell Help Assists in CD8$^+$ T Cell Priming

Finally, we considered how these insights into how T cell help works could help explain the puzzling observations that T cell help can be mediated through alternative cytokines in different models of CD8$^+$ T cell priming (Filatenkov et al., 2005; Oh et al., 2008; Wiesel et al., 2010). As the nature of the innate stimuli associated with the respective antigenic challenge likely differs substantially between the different models of CD8$^+$ T cell priming, and our findings above showed that T cell help was not uniquely linked to a single innate circuit (i.e., IFN-α/β-IL-15 axis), we reasoned that CD4$^+$ T-cell-dependent amplification might also apply to innate pathways elicited through other danger signals. This was a particularly compelling notion, as it implied that the type of innate stimulus determined how T cell help was mediated, rather than the CD4$^+$ T cells themselves selecting the downstream signals that aid CD8$^+$ T cell priming. To test this hypothesis in vivo, we exposed mice again to cell-associated OVA, but this time also included a group in which we administered LPS instead of Poly(I:C), creating a situation in which CD8$^+$ T cell priming in response to the same source of antigen was associated with stimulation of different innate pathways. In agreement with our earlier work (Bennett et al., 1998), LPS-adjuvanted cell-associated OVA induced a helper-dependent antigen-specific CD8$^+$ T cell response (Figure 6B). Strikingly, although the mice were exposed to the same source of antigen, stimulation of different innate pathways at the time of priming changed the cytokine requirements of the ensuing helper-dependent OVA-specific CD8$^+$ T cell response. Here, the Poly(I:C)-adjuvanted, helper-dependent OVA-specific CD8$^+$ T cell response required IL-15, but not IL-12p40 (Figure 6C), whereas the presence of LPS changed this profile so that OVA-specific CD8$^+$ T cell priming no longer required IL-15, but was now dependent on IL-12p40 (Figure 6B). These experiments demonstrated that T cell help can be associated with different cytokine responses in the one model system and highlighted that the innate stimulus determined how CD4$^+$ T cells aided in CD8$^+$ T cell priming.

DISCUSSION

This study has investigated how CD4$^+$ T cells improve the capacity of DCs to prime antigen-specific CD8$^+$ T cell responses. Our comprehensive in vivo analysis of mice infected with HSV-1 revealed an intriguing scenario in which CD8$^+$ DCs required stimulation by both IFN-α/β and CD4$^+$ T cells to produce the IL-15 that naive CD8$^+$ T cells responding to cognate antigen required for their effector differentiation (Figure 5E). In showing that the provision of T cell help in the absence of innate signals failed to elicit IL-15 by DCs and that the helper dependence of OVA-specific CD8$^+$ T cell priming, but not its requirement for IL-15, could be reversed by increasing the strength of innate

stimulation, our data indicate that T cell help amplified the capacity of IFN-α/β to elicit IL-15 in DCs when the IFN-α/β signal alone was ineffective to achieve this. Importantly, $CD4^+$ T-cell-mediated amplification of IFN-α/β signals was not exclusively linked to the provision of IL-15, as CD40 stimulation also facilitated and enhanced the provision of other mediators that are important for T cell help, namely CCL4, CCL5, and IL-6 (Castellino and Germain, 2007; Castellino et al., 2006; Crawford et al., 2011). Together with our observation that altering the innate stimulus delivered alongside the same antigen changed the cytokine requirement of the ensuing helper-dependent antigen-specific $CD8^+$ T cell response, these findings demonstrate an inextricable link between T cell help and innate immune stimulation of DCs. Taken together, this study supports a model of T cell help, in which $CD4^+$ T cells amplify the capacity of DCs to respond to the innate signals generated by the particular environment in which they have encountered antigen. This is an important advance from current models (Bevan, 2004; Wiesel and Oxenius, 2012), as it illustrates that the innate signals, and not the $CD4^+$ T cells themselves, determine the downstream cytokines that ultimately "help" $CD8^+$ T cells. Such an "amplification model" of T cell help is consistent with the well-established observation that priming of $CD8^+$ T cell responses under conditions of weak inflammation is particularly helper dependent. However, rather than $CD4^+$ T cells substituting for ineffective or absent inflammatory and/or microbial stimuli, our data indicate that innate signals still play a crucial role in helper-dependent responses. While these signals alone are likely suboptimal and therefore require $CD4^+$ T-cell-mediated amplification, they are nevertheless crucial as they dictate the resulting cytokine responses that support $CD8^+$ T cell priming. This view on T cell help aligns with a previous report showing that CD40-stimulated $CD8^+$ DCs only secreted IL-12 in vivo when the DCs were co-exposed to microbial products (Schulz et al., 2000). Our findings also substantiate previous speculations that T cell help might be linked to the modulation of danger signals (Castellino and Germain, 2007; Macagno et al., 2007; Schulz et al., 2000). Overall, the present study highlights that the amplification of innate signals by $CD4^+$ T cells represents an important functional aspect of T cell help that likely co-exists with additional DC-dependent or DC-independent mechanisms of T cell help.

In showing that the cytokine requirement of helper-dependent $CD8^+$ T cell priming can be changed by altering the inflammatory stimulus provided at the time of antigen challenge, this study provides an answer to the puzzling question of how T cell help for different $CD8^+$ T cell responses could be mediated through alternative cytokines (Filatenkov et al., 2005; Oh et al., 2008; Wiesel et al., 2011). Considering that allograft responses (Filatenkov et al., 2005) and adenoviral vector immunization (Oh et al., 2008) will most likely be associated with different sets of danger signals, our data predict that $CD4^+$ T cells simply amplified alternative innate circuits in DCs and therefore differed in the way they aided in $CD8^+$ T cell priming. In the case of the allograft response (Filatenkov et al., 2005), $CD4^+$ T cells likely facilitated the provision of IL-12 by DCs, whereas they appear to have promoted DCs to deliver IL-15 following adenoviral vector vaccination (Oh et al., 2008). Accordingly, this explains how T cell help can appear to vary in nature, yet be mediated by the same mechanism. The importance of a flexible system that allows $CD4^+$ T cells to amplify differing cytokine responses is further emphasized by our observations that $CD8^+$ T cell priming required either IL-12p40 or IL-15, but not both at the same time. When interpreted in the context of reports showing that primary $CD8^+$ T cell responses against *Listeria monocytogenes* required IL-12p40 (Oxenius et al., 1999), but not IL-15 (Sandau et al., 2010), while $CD8^+$ T cell priming upon HSV-1 infection, as shown here, or in response to vesicular stomatitis virus infection depended on IL-15 (Schluns et al., 2002), but not IL-12 (Keppler et al., 2012; Sandau et al., 2010), it is possible that these distinct cytokine requirements of primary $CD8^+$ T cell responses could be related to DCs encountering bacteria or viruses, respectively. It is also conceivable that these differential priming requirements could be associated with changes in the relative responsiveness of the T cells to the cytokines induced by the specific innate stimulus. Whatever the underlying reasons for this variability in the priming requirements of $CD8^+$ T cells, the fact that different innate pathways can support $CD8^+$ T cell priming (Ahonen et al., 2004) further supports our proposal that T cell help provides a flexible means through which varying innate circuits can be amplified.

By demonstrating that T cell help amplifies innate signals, our work identifies an important mechanism through which $CD4^+$ T cells endow DCs with optimal capacities to prime $CD8^+$ T cells when danger signals alone are insufficient to achieve this. This is highly relevant to $CD8^+$ T cell priming in infections where microbial immune evasion strategies (Bedoui et al., 2010) or restriction of pathogen replication to peripheral tissues (van Lint et al., 2004) limit the availability of danger signals at sites of T cell priming. Similarly, T-cell-help-induced amplification of innate pathways likely contributes to tumor-specific $CD8^+$ T cell priming, which often is associated with limited exposure to danger signals. However, as indiscriminate amplification of innate circuits in DCs and the resulting facilitation of $CD8^+$ T cell priming can also be dangerous, particularly when it occurs in DCs presenting self or environmental antigens, a sufficiently high activation threshold needs to be imposed on $CD4^+$ T-cell-induced amplification of innate signals. This is likely achieved by restricting the provision of T cell help to cognate interactions between the $CD4^+$ T cell and the DC (Bennett et al., 1998; Smith et al., 2004). Defining T cell help therefore as an antigen-specific means through which innate stimulation of DCs can be regulated could be interpreted as a checkpoint system, in which DCs acquiring antigen in the context of limited danger signals "seek" validation from cognate $CD4^+$ T cells. Only if the additional indirect information obtained through this validation step is successfully integrated into the process (i.e., concurrent access of the DC to MHC-II restricted epitopes; presence and appropriate spatiotemporal activation of cognate $CD4^+$ T cells) will the innate stimuli be amplified to levels that support $CD8^+$ T cell priming by the DC.

In summary, the present study has demonstrated an inextricable association between innate signals and T cell help through which antigen-specific control of innate circuits regulates how DCs respond to danger signals and subsequently gain the capacity to prime $CD8^+$ T cell responses. These insights not only have important implications for our conceptual view on T cell help but

also further highlight the proposal that DC maturation should not be viewed as a binary "on-off" event (Reis e Sousa, 2006), but rather as a process in which the integration of multiple signal qualities (Macagno et al., 2007) determines the overall capacity of a DC to evoke effector differentiation of T cells (Joffre et al., 2009).

EXPERIMENTAL PROCEDURES

Mice

C57BL/6, *H2-ab1*$^{-/-}$, *Il15*$^{-/-}$, *Il12b*$^{-/-}$, *Ifnar2*$^{-/-}$, *Cd40*$^{-/-}$, *Cd40lg*$^{-/-}$, CD45.1^{+} gBT-I, gBT-I on an *Ifnar2*$^{-/-}$ background (*Ifnar2*$^{-/-}$ gBT-I), and CD11c DTR mice were bred and maintained at the Department of Microbiology and Immunology at the University of Melbourne animal facility. The University of Melbourne Animal Ethics Committee approved all animal experiments.

Viruses and Virus Infection

Wild-type HSV-1 KOS was grown using Vero cells (CSL). Mice were infected epicutaneously with 10^6 plaque-forming units of HSV-1 as previously described (van Lint et al., 2004). Some mice were treated on days −3 and −1 before infection or antigen challenge with 100 μg anti-CD4 mAb (GK1.5, WEHI recombinant antibody facility, Melbourne) intraperitoneally.

Preparation of Cell-Associated OVA

For the preparation of cell-associated OVA, splenocytes from C57BL/6 mice were isolated in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), washed, and γ irradiated (1,500 rads) as previously described (Bennett et al., 1998). The cells were then washed once prior to incubation with 10 mg/ml OVA protein in Hank's balanced salt solution (HBSS) for 10 min at 37°C. After three subsequent washes the cells were filtered and 2 × 10^7 cells were injected per mouse intravenously in 0.2 ml of HBSS. In some experiments, cell-associated OVA was co-administered with Poly(I:C) (0.05 μg–5 μg/mouse; InvivoGen) or LPS (1 μg/mouse; Difco, derived from *E. coli* 0111:B4).

CD8^{+} DCs Isolation

Single-cell suspensions from naive spleen or brachial lymph nodes of mice infected with HSV-1 2 days earlier or from pooled inguinal, cervical, and brachial LN of naive C57BL/6 mice were enriched for conventional DCs (excluding plasmacytoid DCs) using antibodies against Gr-1 (RB6-8C5), CD3ε (KT3), CD19 (ID6), Thy1 (T24), B220 (RA36B2), and erythrocytes (Ter-119) and magnetic bead depletion as previously described (Bedoui et al., 2009). Purity of the enriched cell suspension usually yielded between 60% and 85% CD11c^{+} cells. Cells were stained with antibodies against CD11c (N418), CD8 (53-6.7), XCR1 (ZET, Biolegend), CD3 (145-2C11, BD Biosciences), CD19 (1D3), NK1.1 (PK136), CD40 (MR1), IA/E (2G9), and IL-15Rα (FAB551F). CD8^{+} DCs were either processed on an analytic flow cytometer (FACS Canto or LSR Fortessa BD Biosciences) or flow cytometrically sorted (FACS Aria, BD Biosciences) based on the following characteristics: CD11c^{hi}, MHC-II$^{hi/int}$, XCR1^{+}, and CD8^{+}. Sorted CD8^{+} DCs from the spleen were used for real-time PCR (Figure 5B). CD8^{+} DCs sorted from brachial lymph nodes of HSV-1-infected mice (Figure 1F) were co-cultured with CFSE-labeled gBT-1 cells for 60 hr. Purity of sorted CD8^{+} DCs routinely exceeded 94%.

Flow Cytometry Analysis of Endogenous, Virus-Specific CD8^{+} T Cells

Anti-viral CD8^{+} T cells specific for $gB_{498–505}$ from HSV-1 were measured in the spleen or lymph nodes using H-2K^{b}-restricted $gB_{498–505}$-specific tetramers. After surface staining with anti-CD8 mAb (53.6-7), anti-CD44 mAb (IM7), and tetramer staining, viable CD8^{+} CD44^{+} tetramer^{+} cells were determined by flow cytometry. Intracellular $gB_{498–505}$-induced IFN-γ production was determined as previously described (Smith et al., 2004).

Preparation and Proliferation Analysis of Virus-Specific Transgenic T Cells

gBT-I and *Ifnar2*$^{-/-}$ gBT-I were purified as described (Bedoui et al., 2009). In brief, T cells isolated from lymph nodes and spleens were enriched by incubation for 30 min with antibodies against Mac-1 (M1/70), F4/80 (F4/80), erythrocytes (TER-119), Gr-1 (RB6-8C5), I-A/E (M5114), and CD4 (GK1.5) for CD8^{+} T cell enrichment. Cells binding the antibodies were removed with goat anti-rat IgG-coupled magnetic beads (QIAGEN). Purity of the enrichment was routinely 90%–95% as determined by flow cytometry. Enriched naive gBT-I were intravenously transferred into mice 1 day before infection. In some experiments, enriched T cells were further processed for labeling with 2.5 μM CFSE (Sigma) to be used in ex vivo antigen presentation assays. For this, 5 × 10^4 CFSE-labeled gBT-I were co-cultured with graded concentrations of purified CD8^{+} DCs from HSV-1 infected mice for 60 hr. Proliferation was measured by CFSE dilution of CD8^{+} T cells using a flow cytometer.

Bone Marrow Chimeras

Mixed chimeras were generated as previously described (Davey et al., 2010). In brief, C57BL/6 or congenic CD45.1 mice were lethally irradiated with 2 × 550 cGy and reconstituted with a 1:1 mix of 2.5 × 10^6 T-cell-depleted bone marrow cells from *Ifnar2*$^{-/-}$ or *Il15*$^{-/-}$ mice and transgenic CD11c DTR. Chimeric mice were allowed to reconstitute for at least 8 weeks. Depletion of CD11c^{+} cells was achieved by injecting mixed chimeras containing BM from CD11c DTR twice with 100 ng of DTx on days −3 and −1 via the intraperitoneal route before analysis.

In Vitro Generation of eCD8^{+} DCs

For in vitro generation of murine bone-marrow-derived DCs, bone marrow from B6 mice was cultured in the presence of flt3L as described previously (Naik et al., 2005). Bone marrow cells were centrifuged once and resuspended in 1 ml of RBC lysis buffer (Sigma-Aldrich) for 15 s. Cells were cultured at 1.5 × 10^6/ml in complete Media supplemented with glutamax, FCS, antibiotics, and 100 ng/ml human FLt3L (BioXCell) for 8 days at 37°C. Then cultures were sorted on the basis of PDCA1, SIRPα, CD11c, CD11b, and CD24 in accordance with previous work (Lauterbach et al., 2010). 5 × 10^5 CD11b^{lo} SIRPα^{lo} CD24^{high} DCs (eCD8^{+} DCs) were stimulated for 4 hr with IFN-α A (PBL) in the presence or absence of anti-CD40 mAb.

Cytokine and Chemokine Determination

Supernatants were harvested from sorted flt3L DC cultures 4 hr post stimulation and assessed for CCL3, CCL4, CCL5, IL-6, and IL-12/23p40 by BD cytometric bead array kits according to manufacturer's instructions. Samples were assessed using a LSR Fortessa with all concentrations determined relative to a standard curve.

Real-Time PCR

For quantitative real-time PCR analysis, RNA was extracted using RNeasy Micro Kit (QIAGEN) and cDNA synthesized with SuperScript III Reverse Transcriptase (Invitrogen) using oligo-dT primers (Promega). Real-time PCR was performed with Fast SYBR green Master mix (Life Technologies) with primers for *IL-15* (5′-TTA CGC GCT GCA GGG ACC-3′ and 5′-AGG GCC ATG TGT CAA GGT GG-3′) and *Gapdh* (5′-CCA GGT TGT CTC CTG CGA CTT-3′ and 5′-CCT GTT GCT GTAG CCG TAT TCA-3′). *Il15* mRNA expression was normalized to *Rn18s* (Figure 4A) or *Gapdh* (Figure 5) and relative expression (RE) was determined (RE = $2^{-(\Delta\Delta CT)}$).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.12.058.

AUTHOR CONTRIBUTIONS

M.G., P.G.W., W.R.H., and S.B. conceived of the study and designed and performed experiments; S.B. wrote the manuscript; M.G., A.T.S., and C.T. performed real-time PCR; G.M.D. and A.B. performed experiments and

coordinated mouse breeding; J.D.M. and S.J.T. analyzed effector functions, T.G. and R.A.S. contributed intellectually and critically reviewed the manuscript; and M.O. performed flt3L culture experiments and provided crucial reagents and intellectual input.

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